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(71) Applicant *(for all designated States except US)*: **FIDIA ADVANCED BIOPOLYMERS S.R.L.** [IT/IT]; Via Ponte della Fabbrica 3/B, I-35031 Abano Terme (IT).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **BELLINI, Davide** [IT/IT]; Via Po, 34, I-35036 Montegrotto Terme (IT). **CRESCENZI, Vittorio** [IT/IT]; Via Andrea Pitti, 27, I-00147 Roma (IT). **FRANCESCANGELI, Andrea** [IT/IT]; Via Paolo Segneri, 14, I-00152 Roma (IT).

(74) Agent: **GERVASI, Gemma**; Notarbartolo & Gervasi S.P.A., Corso di Porta Vittoria, 9, I-20122 Milano (IT).

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(54) Title: PERCARBOXYLATED POLYSACCHARIDES, AND A PROCESS FOR THEIR PREPARATION

(57) Abstract: The present invention relates to percarboxylated polysaccharide selected from the group consisting of gellan, carboxymethylcellulose, pectic acid, pectin and hyaluronic acid derivatives; the process for their preparation and their use in the pharmaceutical, biomedical, surgical and healthcare fields.

PERCARBOXYLATED POLYSACCHARIDES, AND A PROCESS FOR THEIR PREPARATION

Field of the invention

The present invention describes percarboxylated polysaccharides, the process for their preparation and their use in the pharmaceutical field and in the preparation of biomaterials for surgical, biomedical and healthcare uses.

State of the art

Hyaluronic acid is a heteropolysaccharide composed of alternating residues of D-glucuronic acid and N-acetylglucosamine. It is a polymer with a linear chain and a molecular weight that may vary between 50,000 and 13,000,000 Da, according to the source from which it is obtained and the methods used to prepare it.

It is present in nature in the pericellular gels, in the fundamental substance of the connective tissue of vertebrate organisms, of which it represents one of the main components, in the synovial fluid of joints, in the vitreous humor, in the tissues of human umbilical cords and in cockscombs.

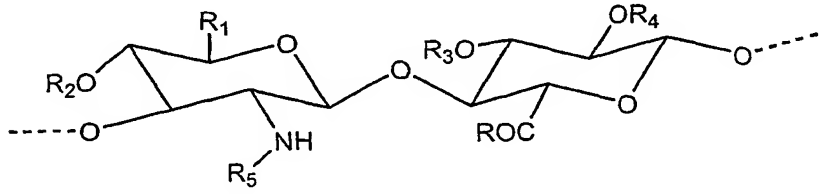
In recent years, various polysaccharides, not carboxylated by oxidation of the primary hydroxyls, have been modified by the use of a selective reagent, specific to primary alcohol groups, namely 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO). The modification of non-carboxylated polysaccharides such as pullulan (De Nooy A.E.J. et al., *Macromolecules* 1996, 29, 6541-6547) methyl-α-D-glycopyranoside (De Nooy A.E.J. et al., *Tetrahedron* 1995, 51, 8023-8032), scleroglucan (De Nooy A.E.J. et al., *Carbohydrate Research* 2000, 324, 116-126), have been described in a publication entitled "Selective oxidation of primary alcohol groups in polysaccharides" (1997) by De Nooy A.E.J., which reports various oxidation processes both on non-carboxylated polysaccharides and, more generally, on hydroxy groups of alcohols of a different nature. However, although the TEMPO-mediated oxydation of hyaluronic acid is known to the state of the art (Bo Jiang et al., *Carbohyd. Res.* 2000; vol. 327, pages 455-61), there is no report in the art on the application of such reaction on carboxylated polysaccharides. Concerning this, it is worthy to note that Jiang et al. themselves affirmed in the above document that the TEMPO-mediated oxidation is a rather complex process, and consequently the presence of other groups on the hyaluronic chain, as occurs for

hyaluronic acid derivatives, may also have unforeseeable repercussion on the reaction. Moreover, following to the reaction a substantial degradation of the polymer occurred, that is caused, according to Jang et al., by the oxidation process itself.

The introduction of additional carboxy groups in the hyaluronic chain increases the stability of the derivatives (increase in the percentage of esterification, amidation, cross-linking, etc.), their viscosity, their hydrophilic properties (the greater the number of carboxylate groups, the greater their solubility in water and in aprotic solvents, such as DMSO), and their hydrophobic properties (the introduction of additional carboxy groups makes it possible to introduce lipid molecules, such as long-chain fatty acids, by esterification or amidation).

Summary of the invention

The present invention relates to "percarboxylated" hyaluronic acid derivatives, comprising at least one repeating unit of formula (I):



(I)

wherein R is OH, O^-, an alcoholic or an amino group of the aliphatic, aromatic, arylaliphatic, cycloaliphatic and heterocyclic series;

R_1 is COR_6, wherein R_6 is OH, O^-, an alcoholic or an amino group of the aliphatic, aromatic, arylaliphatic, cycloaliphatic and heterocyclic series; an alcoholic group of hyaluronic acid, or an amino group of N-deacetylated hyaluronic acid;

R_2, R_3, R_4, equal or different from each other, are H, SO_3^-, an acyl group deriving from a carboxylic acid of the aliphatic, aromatic, arylaliphatic, cycloaliphatic, heterocyclic series; or a residue of hemiester of succinic acid or of heavy metal salts of hemiester of succinic acid;

R_5 is $COCH_3$, H, SO_3^-, an acyl group deriving from a carboxylic acid of the aliphatic, aromatic, arylaliphatic, cycloaliphatic, heterocyclic series, or an acyl group of hyaluronic acid;

provided that, when R is OH, R_5 is $COCH_3$ and $R_2 = R_3 = R_4 = H$, R_6 is different

from OH and O^-.

Further subject of the invention are "percarboxylated" polysaccharides selected from the group consisting of gellan, carboxymethylcellulose, pectin and pectic acid.

The present invention also relates to the process for their preparation and the numerous applications of such products in the pharmaceutical, biomedical, surgical and healthcare fields.

Detailed description of the invention

The term "percarboxylated", as used herein, means that all or part of the primary hydroxyl groups present on the polymer have been replaced by carboxy groups, and modifications thereof, by an oxidation process.

The term "percarboxylation degree" as used herein, means the percentage of carboxy groups, or modifications thereof, introduced by an oxidation process.

According to the invention, the percarboxylation degree of the present derivatives is comprised between 1% and 100%, and preferably between 25% and 75%.

The present invention relates to new "percarboxylated" polysaccharides selected from the hyaluronic acid derivatives of formula (I) above reported, gellan, carboxymethylcellulose, pectin and pectic acid.

Of the percarboxylated derivatives of hyaluronic acid according to the present invention, the following are to be preferred:

- the hyaluronic acid esters wherein a part or all of the carboxy functions, including those obtained by oxydation of the primary hydroxyls, are esterified with alcohols of the aliphatic, aromatic, arylaliphatic, cycloaliphatic, heterocyclic series, as disclosed in EP 0216453 B1, which we incorporate herewith by reference;
- the autocross-linked esters of hyaluronic acid wherein part or all of the carboxy functions, including those obtained by oxydation of the primary hydroxyls, are esterified with the alcohol functions of the same polysaccharide chain or other chains, as disclosed in EP 0341745 B1, which we incorporate herewith by reference;
- the cross-linked esters of hyaluronic acid wherein part or all of the carboxy functions, including those obtained by oxydation of the primary hydroxyls, are esterified with polyalcohols of the aliphatic, aromatic, arylaliphatic, cycloaliphatic,

heterocyclic series, generating cross-linking by means of spacer chains, as disclosed in EP 0265116 B1, which we incorporate herewith by reference;

- the hemiesters of succinic acid or the heavy metal salts of the hemiester of succinic acid with hyaluronic acid or with partial or total esters of hyaluronic acid, like those disclosed in WO 96/357207, which we incorporate herewith by reference;

- the O-sulphated derivatives, as disclosed in WO95/25751, which we incorporate herewith by reference, or N-sulphated derivatives, as disclosed in WO98/45335, which we incorporate herewith by reference;

- the amides of hyaluronic acid, like those disclosed in WO00/01733, which we incorporate herewith by reference.

Preferred are the benzyl ester of hyaluronic acid having a percarboxylation degree of 25%, the zinc salt of hyaluronic acid having a percarboxylation degree of 25 % and the autocross-linked hyaluronic acid (ACP) having a percarboxylation degree of 50%.

When not otherwise specified, the terms aliphatic, aromatic, arylaliphatic, cycloaliphatic and heterocyclic, as used herein, should be intended as follows:

- "aliphatic" means acyclic or pertaining to open-chain or branched carbon compounds such as alkanes, alkenes or alkynes. Examples of an aliphatic moiety include but are not limited to C1-C20 noncyclic hydrocarbons and their isomers such as methyl, ethyl, propyl, isopropyl, n-butyl, tert-butyl, pentyl, isopentyl, neopentyl, tert-pentyl, 2-methylbutyl, 1,2-dimethylpropyl, hexyl, isohexyl, 1-methylpentyl, 2-methylpentyl, 3-methylpentyl, 1,1-dimethylbutyl, 1,2-dimethylbutyl, 2,2-dimethylbutyl, 1,3-dimethylbutyl, 2,3-dimethylbutyl, 3,3-dimethylbutyl, 1,2-ethylbutyl, 2-ethylbutyl, 1,1,2-trimethylpropyl, heptyl, octyl, nonyl, decyl, undecyl, dodecyl, tridecyl, tetradecyl, pentadecyl, hexadecyl, cetyl, heptadecyl, octadecyl, nonadecyl, stearyl, etc.

- "aromatic" means an aryl moiety having one or more unsaturated rings, each ring usually having 5 to 8 members and preferably 5 to 6 members. Examples of the aromatic moiety include but are not limited to benzyl, toluyl, naphalyl, anthracenyl, phenantryl, fluorenyl, coronenyl, triphenylenyl, fluoranthenyl, benzofluoranthenyl, benzopyrenyl and pyrenyl.

- "cycloaliphatic" indicates a carbon ring structure, usually having 3 to 8 members and preferably 5 to 6 members, that does not contain a resonance structure. Examples of cycloaliphatic groups include but are not limited to cycloalkanes and cycloolefins such as cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, cyclohexenyl (tetrahydrobenzenyl), cyclohexylidenyl, and cyclooctadienyl.

- "heterocyclic" relates to dissimilar atoms in a ring. A heterocyclic group is a heteroaryl group usually having a 3- to 8-membered, preferably 5- to 6-membered ring or fused ring containing at least one hetero atom (such as O, S, N, etc.) and include but are not limited to thienyl, furanyl, pyranyl, 2H-pyrrolyl, imidazolyl, pyrazolyl, pyridyl, pyrazinyl, pyrimidyl, pyridazinyl, isothiazolyl, isoxazolyl, furazanyl, benzothienyl, isobenzofuranyl, chromenyl, indolindinyl, isoindolyl, indolyl, purinyl, quinolidinyl, isoquinolyl, quinolyl, phtalazinyl, quinazolyl, carbazolyl, acridinyl, and phenanthridinyl.

- "arylaliphatic" means a group having both aromatic and aliphatic substituents as defined above. Examples of arylalkyl groups include but are not limited to ethylbenzenyl, isobutylbenzeneyl, benzyl, ethylbenzyl, propylbenzyl, isopropylbenzyl, butylbenzyl, isobutylbenzyl, cyclohexylbenzyl, styrenyl, and biphenyl.

Percarboxylated hyaluronic acid derivatives can be used for the preparation of pharmaceutical compositions, for example in the form of gels, for the transport and release of drugs and biologically active substances to be used in viscoelastic surgery or ophthalmic surgery.

The present percarboxylated derivatives can also by salified with heavy metals wherein the heavy metals are the elements of the 4^{th}, 5^{th} or 6^{th} period of the periodic table, such as silver, iron, cobalt, copper, zinc, arsenic, strontium, zirconium, antimony, gold, cesium, tungsten, selenium and platinum, ruthenium, bismuth, tin, titanium, gallium, mercury.

These salts can be used in dermatology, ophthalmology, dentistry, rheumatology, urology, gynaecology, internal surgery, as food supplements, antioxidants, antirheumatic and anticancer agents, antiinflammatories, analgesics and antiulcer agents.

Also the salts of the present percarboxylated derivatives may be prepared with pharmacologically and/or biologically active substances.

Of the pharmacologically active substances, the following are preferred:

antibiotics, anti-infective, antimicrobial, antifungal, antiviral, cytostatic, cytotoxic, anticancer, anti-inflammatory, wound-healing agents, anaesthetics, analgesics, vasoconstrictors, cholinergic or adrenergic agonists and antagonists, antithrombotics, anticoagulants, haemostatic, fibrinolytic, thrombolytic agents.

As biologically active substances should be intended for example proteins and their fragments, peptides, polynucleotides, growth factors, enzymes, vaccines, or substances used in the treatment of diseases associated with genetic defects, such as those that are caused by enzymatic hypo- or hyperactivity due to defects of the gene that codes for a given enzyme, or deforming or hereditary diseases.

The present percarboxylated derivatives can also be used in association with radioactive and non-radioactive substances used in contrast systems, and as tracers in *in vivo* diagnostics for the identification and cure of cancer tissues or damaged tissues.

A considerable advantage is represented by the possibility of processing the compounds of the present invention and their salts in various forms of biomaterials such as sponges, films, membranes, threads, tampons, non-woven tissues, felts, microspheres, nanospheres, gauze pads, gels, guide channels, and associations thereof.

These biomaterials may be constituted by one or more of the present percarboxylated derivatives, optionally in association with natural, synthetic, semisynthetic polymers and, optionally, further being in combination with pharmacologically and/or biologically active substances.

Examples of the natural polymers that can be used are collagen, coprecipitates of collagen and glycosaminoglycans, cellulose, polysaccharides in the form of gels such as chitin, chitosan, pectin and pectic acid, agar, agarose, xanthane, gellan, alginic acid or the alginates, polymannan or polyglycans, starch, natural gums.

Examples of semisynthetic polymers of possible use are collagen cross-linked with agents such as aldehydes or precursors of the same, dicarboxylic acids or their halides, diamine, derivatives of cellulose, hyaluronic acid, chitin or chitosan,

xanthane, pectin or pectic acid, polyglycans, polymannan, agar, agarose, natural gums and glycosaminoglycans.

Synthetic polymer can be chosen, for example, from the group consisting of polylactic acid, polyglycolic acid or the derivatives thereof, polydioxanes, polyphosphazenes, polysulphonic resins, polyurethanes, PTFE.

The above-said biomaterials can be used in various surgical fields, for example in internal surgery, osteo-articular surgery, neurosurgery, anastomotic, viscoelastic, ophthalmic, oncological, plastic-aesthetic, otolaryngological, abdominal-pelvic, urogynaecological, cardiovascular surgery, in the prevention of post-surgical adhesions and hypertrophic scars.

Moreover, they can be used in blood dialysis and in other branches of medicine such as cardiology, angiology, dermatology, ophthalmology, otolaryngology, dentistry, orthopaedics, gynaecology, urology, in extracorporeal circulation and oxygenation and in cosmetics.

Said biomaterials, in their various forms, are particularly suitable for use as scaffolds for the growth of cells such as mesenchymal or mature cells to obtain connective, bone, glandular, nervous, muscular, hepatic tissue etc.

The biomaterials comprising the present percarboxylated derivatives can be used, in association with biologically and/or pharmacologically active substances, as vehicling agents for the preparation of slow release pharmaceutical compositions; moreover, the present percarboxylated derivatives can be used as the active ingredients, in combination with pharmaceutically acceptable excipients and/or diluents, for the preparation of pharmaceutical compositions.

The derivatives thus obtained can also be used in the processes of coating objects used both in the medical field and in other sectors of industry, providing new biological characteristics to the surfaces of the materials used as supports.

The objects that can be thus coated are, for example, catheters, guide channels, probes, cardiac valves, soft tissue replacements, replacements of animal origin, artificial tendons, bone and cardiovascular replacements, contact lenses, blood oxygenators, artificial kidneys, hearts, pancreas and livers, blood bags, syringes, surgical instruments, filtration systems, laboratory instruments, containers for cell and tissue culture and regeneration, supports for peptides, proteins and

antibodies.

The process of coating the surfaces of such objects can be performed by the Plasma Coating technique, as described in the international patent application by the Applicant, No. WO96/24392.

The present percarboxylated derivatives can be obtained by an oxidation process, that acts selectively on the primary hydroxyl groups, for example by reaction of the polisaccharide, selected from hyaluronic acid and derivatives thereof, gellan, carboxymethylcellulose, pectic acid and pectin, with sodium hypochlorite in aqueous solution at a low temperature, preferably ranging between 0°C and -1°C, and in the presence of 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO). The degree of percarboxylation deriving therefrom, depends on the quantity of the oxidising agent (hypochlorite) used in the reaction.

Also when the starting polysaccharide is a hyaluronic acid derivative, the present process has lead to the desired percarboxylated products, without showing the drawbacks expected, and in particular the substantial degradation of the polymer disclosed by Bo Jiang et al.

The process for the preparation of percarboxylated hyaluronic acid derivatives wherein R_6 is different from OH or O^-, comprises the following steps:

a) selective oxidation of part or all primary hydroxyl groups of the starting hyaluronic acid derivatives;

b) preparation of the quaternary ammonium salt, preferably the tetrabutylammonium salt, of the percarboxylated hyaluronic acid derivative coming from step a);

c) reaction of the said quaternary ammonium salt coming from step b) with different reagents depending on which is the derivative desired. In particular, the quaternary ammonium salt is reacted with a sulphating agent to obtain the O-sulphated or N-sulphated derivatives, with chloromethylpyridinium iodide to obtain the autocross-linked derivatives, with succinic anhydride to obtain the hemiesters of succinic acid, etc., as disclosed in the above patent documents, herewith incorporated by reference.

The following examples are given to provide non-limiting illustrations of the present invention.

EXAMPLE 1

Preparation of percarboxylated hyaluronic acid in the form of a sodium salt with 25% percarboxylation

1 g of sodium hyaluronate (2.5 mmole) with a mean molecular weight of 200 KDa is solubilised in 50 ml of water. The solution is cooled in a bath with a thermostat set at –1°C, and is then supplemented with 40 mg (0.26 mmole) of TEMPO and 260 mg (2.5 mmole) of NaBr (sodium bromide). A solution of 15% sodium hypochlorite is added in a quantity of 0.3 ml, equal to 0.625 mmole, corresponding to 25% of the moles of sodium hyaluronate present. The solution is mixed and the pH maintained at 9-9.2 by the addition of a solution of 0.5 M NaOH. Some minutes later, the pH remains stable with no further additions of this kind. A volume of absolute ethanol equal to 5 ml is added and the solution is neutralised by adding 1 M HCl until the pH reaches 6.9 – 7.2. Then 95 mg (2.5 mmoles) of sodium borohydride is added and the mixture is agitated overnight at room temperature. The solution is neutralised to pH 6 and precipitated with acetone.

The product thus obtained is characterised analytically to determine the percentage of carboxylation.

Yield of the reaction: 95%.

Percentage of percarboxylation (acidimetric): 25%.

EXAMPLE 2

Preparation of percarboxylated hyaluronic acid in the form of a sodium salt with 50% percarboxylation

1 g of sodium hyaluronate (2.5 mmoles) with a mean molecular weight of 200 KDa is solubilised in 50 ml of water. The solution is cooled in a bath with a thermostat set at –1°C, and is then supplemented with 40 mg (0.26 mmole) of TEMPO and 260 mg (2.5 mmole) of NaBr (sodium bromide). A solution of 15% sodium hypochlorite is added in a quantity of 0.6 ml, equal to 1.25 mmole, corresponding to 50% of the moles of sodium hyaluronate present. The solution is mixed and the pH maintained at 9-9.2 by the addition of a solution of 0.1 M NaOH. Some minutes later, the pH remains stable with no further additions of this kind. A volume of absolute ethanol equal to 5 ml is added and the solution is neutralised by adding 1 M HCl until the pH reaches 6.9 – 7.2. Then 95 mg (2.5 mmoles) of sodium

borohydride is added and the mixture is agitated overnight at room temperature. The solution is neutralised to pH 6 and precipitated with acetone.

The product thus obtained is characterised analytically to determine the percentage of carboxylation.

Yield of the reaction: 95%.

Percentage of percarboxylation (acidimetric): 50%.

EXAMPLE 3

Preparation of percarboxylated hyaluronic acid in the form of a sodium salt with 75% percarboxylation

1 g of sodium hyaluronate (2.5 mmoles) with a mean molecular weight of 200 KDa is solubilised in 50 ml of water. The solution is cooled in a bath with a thermostat set at –1°C, and is then supplemented with 40 mg (0.26 mmole) of TEMPO and 260 mg (2.5 mmole) of NaBr (sodium bromide). A solution of 15% sodium hypochlorite is added in a quantity of 0.9 ml, equal to 1.875 mmole, corresponding to 75% of the moles of sodium hyaluronate present. The solution is mixed and the pH maintained at 9-9.2 by the addition of a solution of 0.5 M NaOH. Some minutes later, the pH remains stable with no further additions of this kind. A volume of absolute ethanol equal to 5 ml is added and the solution is neutralised by adding 1 M HCl until the pH reaches 6.9 – 7.2. Then 95 mg (2.5 mmoles) of sodium borohydride is added and the mixture is agitated overnight at room temperature. The solution is neutralised to pH 6 and precipitated with acetone.

The product thus obtained is characterised analytically to determine the percentage of carboxylation.

Yield of the reaction: 95%.

Percentage of percarboxylation (acidimetric): 75%.

EXAMPLE 4

Preparation of percarboxylated gellan in the form of a sodium salt with 50% percarboxylation

2 g of gellan sodium salt (2.95 mmoles) with a mean molecular weight of 700 KDa is solubilised in 100 ml of water. The solution is cooled in a bath with a thermostat set at –1°C, and is then supplemented with 40 mg (0.26 mmole) of TEMPO and 300 mg (2.9 mmole) of NaBr (sodium bromide). A solution of 15% sodium

hypochlorite is added in a quantity of 0.705 ml, equal to 1.47 mmole, corresponding to 50% of the moles of gellan sodium salt present. The solution is mixed and the pH maintained at 9-9.2 by the addition of a solution of 0.5 M NaOH. Some minutes later, the pH remains stable with no further additions of this kind. A volume of absolute ethanol equal to 5 ml is added and the solution is neutralised by adding 1 M HCl until the pH reaches 6.9 – 7.2. Then 110 mg (2.9 mmoles) of sodium borohydride is added and the mixture is agitated overnight at room temperature. The solution is neutralised to pH 6 and precipitated with acetone.

The product thus obtained is characterised analytically to determine the percentage of carboxylation.

Yield of the reaction: 95%.

Percentage of percarboxylation (acidimetric): 50%.

EXAMPLE 5

Preparation of a benzyl ester (HYAFF®11) from hyaluronic acid with 25% percarboxylation

6.34 g (10 mmoles) of tetrabutylammonium salt of percarboxylated hyaluronic acid according to example 1 is solubilised in 250 ml of dimethylsulphoxide (DMSO) at room temperature. To this solution is added 1187 ml of benzyl bromide (10 mmoles) and the solution is kept at a temperature of 30°C for 24 hours. A solution of 2.5% (w/w) NaCl in water is then added and the resulting mixture is poured into 750 ml of acetone, while agitating. A precipitate is formed that is filtered and washed three times in 100 ml of acetone/water (ratio 5:1), three times in 100 ml of acetone, and then vacuum-dried for 24 hours at 30°C. Thus, 4.25 g of the desired product are obtained with 125% of total benzylic esterification (it should be intended that all the carboxy groups of the polymer not percarboxylated, and all the carboxy groups coming from percarboxylation are esterified). Quantitative determination of the benzylic alcohol content is conducted by gas chromatography after alkaline hydrolysis. The total content of ester groups is determined according to the saponification method described on pages 169-172 of "Quantitative organic analysis via functional groups', IV Ed., John Wiley and Sons Publication.

EXAMPLE 6

Preparation of cross-linked hyaluronic acid (ACP) from hyaluronic acid with 50%

percarboxylation

6.5 g (10 mmoles) of tetrabutylammonium salt of percarboxylated hyaluronic acid according to example 2 is solubilised in 260 ml of N-methyl-2-pirrolidone (NMP) at room temperature. To this solution is added 1.4 ml of triethylamine (10 mmoles) and the resulting solution is agitated for 30 minutes. To this solution is added 0.766 mg of 2-chloro-1-methyl-pyridinium iodide equal to 30% of the initial moles of percarboxylated hyaluronic acid dissolved in 5 ml of NMP. The solution is agitated for 4 hours at room temperature. A saline solution of 2.5% NaCl in water (w/w) is then added. The mixture obtained is slowly poured into 750 ml of acetone while under constant agitation. A precipitate is formed that is filtered and washed three times in 100 ml of acetone/water (ratio 5:1) and three times with 100 ml of acetone, and then vacuum-dried for 24 hours at 30°C. Thus, 3.9 g of the desired product are obtained equal to 30% cross-linking. The total content of ester groups is determined according to the saponification method described on pages 169-172 of "Quantitative organic analysis via functional groups', IV Ed., John Wiley and Sons Publication.

EXAMPLE 7

Preparation of a zinc salt of hyaluronic acid with 25% percarboxylation

2 g of tetrabutylammonium salt of percarboxylated hyaluronic acid according to Example 1 is solubilised in 100 ml of a 5% (w/w) solution of zinc chloride ($ZnCl_2$) in water. The solution is agitated for 15 hours at room temperature. In order to eliminate the excess salts, the solution is dialysed through dialysis membranes until all the residue salts (chlorides) have disappeared. To demonstrate the absence of salts from the dialysed solution, it is tested with a 0.1 molar solution of silver nitrate in water. If the dialysed solution does not become cloudy on contact with the silver nitrate solution, this indicates that no chloride residues are present. The salt-free, dialysed solution is freeze-dried and analysed for its zinc content. The zinc content proves to be 10% bivalent zinc (Zn^{2+}), vs the theoretical content of 7.95. The resulting percentage perfectly reflects the value of percarboxylation of the hyaluronic acid used.

EXAMPLE 8

Preparation of films using percarboxylated hyaluronic acid esters (HYAFF®)

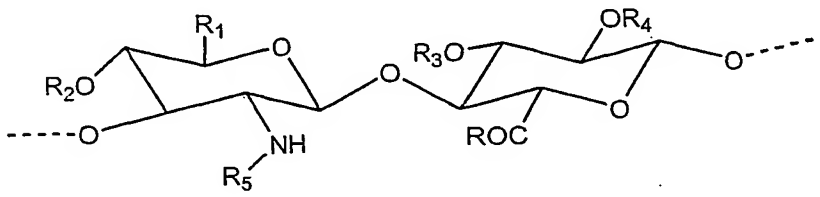
A solution equal to 100 mg/ml of the percarboxylated derivative in dimethylsulphoxide (DMSO) according to Example 5 is prepared by dissolving 1 g of benzyl ester at 125% in 10 ml of DMSO. A thin layer of solution is spread over a glass plate, taking care to create a layer that is 10 times thicker than the desired thickness of the final film. The glass plate is immersed in a bath of ethanol that absorbs the DMSO but does not solubilise the percarboxylated hyaluronic acid ester, which solidifies. The film is detached from the glass plate and washed repeatedly with water and again with ethanol. The film thus obtained is dried in a press for 24 hours at 30°C.

CLAIMS

1. Percarboxylated polysaccharides, having a percarboxylation degree between 1% and 100% and selected from the group consisting of hyaluronic acid derivatives, gellan, carboxymethylcellulose, pectic acid and pectin.

2. Percarboxylated polysaccharides, wherein the said percarboxylation degree is between 25% and 75%.

3. Percarboxylated polysaccharides, wherein the said hyaluronic acid derivative comprises at least one repeating unit of formula (I):



(I)

wherein R is OH, O^-, an alcoholic or an amino group of the aliphatic, aromatic, arylaliphatic, cycloaliphatic and heterocyclic series;

R_1 is COR_6, wherein R_6 is OH, O^-, an alcoholic or an amino group of the aliphatic, aromatic, arylaliphatic, cycloaliphatic and heterocyclic series; an alcoholic group of hyaluronic acid, or an amino group of N-deacetylated hyaluronic acid;

R_2, R_3, R_4, equal or different from each other, are H, SO_3^-, an acyl group deriving from a carboxylic acid of the aliphatic, aromatic, arylaliphatic, cycloaliphatic, heterocyclic series; or a residue of hemiester of succinic acid or of heavy metal salts of hemiester of succinic acid;

R_5 is $COCH_3$, H, SO_3^-, an acyl group deriving from a carboxylic acid of the aliphatic, aromatic, arylaliphatic, cycloaliphatic, heterocyclic series, or an acyl group of hyaluronic acid;

provided that, when R is OH, R_5 is $COCH_3$ and $R_2 = R_3 = R_4 = H$, R_6 is different from OH.

4. Percarboxylated polysaccharides according to any of claims 1-3, alone or in combination with pharmacologically and/or biologically active substances.

5. Percarboxylated polysaccharides according to claim 4, wherein the said

pharmacologically active substances are selected from the group consisting of antibiotics, anti-infective, antimicrobial, antiviral, antifungal, cytostatic, anticancer, anti-inflammatory, wound healing agents, anaesthetics, cholinergic or adrenergic agonists and antagonists, antithrombotics, anticoagulants, haemostatics, fibrinolytics, and thrombolytics.

6. Percarboxylated polysaccharides according to claim 4, wherein the said biologically active substances are selected from the group consisting of proteins and their fragments, peptides and polynucleotides, growth factors, enzymes, vaccines and substances used in the treatment of diseases associated with genetic defects, deforming and hereditary diseases.

7. Percarboxylated polysaccharides according to any of claims 1-4, salified with metals of the 4^{th}, 5^{th} and 6^{th} period of the periodic table of elements.

8. Percarboxylated polysaccharides according to claim 7, wherein the said metals are selected from the group consisting of silver, cobalt, iron, copper, zinc, arsenic, strontium, zirconium, antimony, gold, caesium, tungsten, selenium, platinum, gallium, ruthenium, bismuth, tin, titanium and mercury.

9. A biomaterial comprising at least a percarboxylated polysaccharide as described in claims 1-8, alone or in association with a natural, a semisynthetic or a synthetic polymer and, optionally, further being in association with biologically or pharmacologically active substances.

10. Biomaterial according to claim 9, wherein the said natural polymer is selected from the group consisting of collagen, coprecipitates of collagen, glycosaminoglycans, cellulose, polysaccharides in the form of gels such as chitin, chitosan, pectin or pectic acid, agar, agarose, xanthane, gellan, alginic acid or the alginates, polymannans or polyglycans, starch and natural gums.

11. Biomaterial according to claim 9, wherein the said semisynthetic polymer is selected from the group consisting of collagen cross-linked with agents such as aldehydes or precursors of the same, dicarboxylic acids or their halides, diamine, derivatives of cellulose, hyaluronic acid, chitin or chitosan, xanthane, pectin or pectic acid, polyglycans, polymannan, agar, agarose, natural gums and glycosaminoglycans.

12. Biomaterial according to claim 9, wherein the said synthetic polymer is

selected from the group consisting of polylactic acid, polyglycolic acid or copolymers of the same or their derivatives, polydioxane, polyphosphazenes, polysulphonic resins, polyurethanes and PTFE.

13. Biomaterial according to claim 9, in association with fibrin, and optionally with other biologically active substances, which biomaterial is a surgical glue.

14. Biomaterial according to claim 9, which is a healthcare or surgical article.

15. Biomaterial according to claim 14, wherein the said healthcare or surgical article is selected from the group consisting of microspheres, nanospheres, membranes, sponges, threads, films, gauzes, guide channels, hydrogels, non-woven tissues, felts, and associations thereof.

16. Biomaterial according to claim 9, which is a scaffold for cell cultures.

17. Biomaterial according to claim 9, for use in surgery, haemodialysis, cardiology, angiology, dermatology, ophthalmology, otorhinolaryngology, dentistry, orthopaedics, gynaecology, urology, in extracorporeal blood circulation and oxygenation, and in cosmetics.

18. Biomaterial according to claim 17, wherein said surgery is selected from the group consisting of pelvic, abdominal, spinal, cardiac, vascular, ophthalmic, orthopaedic, otorhinolaryngological and plastic-aesthetic surgery.

19. Biomaterial according to claim 18, for use as a filler in plastic-aesthetic surgery.

20. Biomaterial according to claim 18, for use as substitutes for the vitreous humor in ophthalmology.

21. Biomaterial according to claim 19, for use in the prevention of surgical adhesions of tissues and hypertrophic scars.

22. Use of the biomaterial according to claim 9, in association with biologically and/or pharmacologically active substances, as vehicling agent for the preparation of slow release pharmaceutical compositions.

23. A pharmaceutical composition comprising as the active agent at least one percarboxylated polysaccharides according to claims 1-8, alone or in association with biologically or pharmacologically active substances, in combination with pharmaceutically acceptable excipients and/or diluents.

24. Use of the percarboxylated polysaccharides according to claims 1-8, in

association with radioactive and non-radioactive substances to be used in contrast systems, for the preparation of markers in *in vivo* diagnostics for the identification and treatment of tumoral or damaged tissues.

25. A biomedical object coated with the percarboxylated polysaccharides according to claims 1-8, wherein the said biomedical object is selected from the group consisting of a bypass, a venous catheter, a shunt, a catheter, a guide channel, a probe, cardiac valves, artificial tendons, bone and cardiovascular replacements, contact lenses, soft tissue replacements, replacements of animal origin, blood oxygenators, artificial kidneys, hearts, pancreas and livers, blood bags, syringes, surgical instruments, filtration systems, laboratory instruments, containers for cells and tissues cultures and for the regeneration of cells and tissues, supports for peptides, proteins and antibodies.

26. Healthcare and surgical articles comprising the percarboxylated polysaccharides according to claims 1-8, wherein the said healthcare or surgical articles are selected from the group consisting of microspheres, nanospheres, membranes, sponges, threads, films, gauzes, guide channels, hydrogels, non-woven tissues, felts, and associations thereof.

27. Process for the preparation of percarboxylated polysaccharides as described in claims 1-8, comprising the selective oxidation of part or all the primary hydroxyl groups of a polysaccharide selected from the group selected from hyaluronic acid and derivatives thereof, gellan, carboxymethylcellulose, pectic acid and pectin.

28. Process according to claim 27, wherein the said selective oxidation is carried out by reacting the said polysaccharide with sodium hypochlorite in aqueous solution, in the presence of 2,2,6,6-tetramethyl-1-piperidinyloxy.

29. Process according to claim 27, wherein the said selective oxidation is carried out at a temperature ranging between 0°C and -1°C,

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(71) Applicant *(for all designated States except US)*: **FIDIA ADVANCED BIOPOLYMERS S.R.L.** [IT/IT]; Via Ponte della Fabbrica 3/B, I-35031 Abano Terme (IT).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **BELLINI, Davide** [IT/IT]; Via Po, 34, I-35036 Montegrotto Terme (IT). **CRESCENZI, Vittorio** [IT/IT]; Via Andrea Pitti, 27, I-00147 Roma (IT). **FRANCESCANGELI, Andrea** [IT/IT]; Via Paolo Segneri, 14, I-00152 Roma (IT).

(74) Agent: **GERVASI, Gemma**; Notarbartolo & Gervasi S.P.A., Corso di Porta Vittoria, 9, I-20122 Milano (IT).

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(54) Title: PERCARBOXYLATED POLYSACCHARIDES, AND A PROCESS FOR THEIR PREPARATION

(57) Abstract: The present invention relates to percarboxylated polysaccharide selected from the group consisting of gellan, carboxymethylcellulose, pectic acid, pectin and hyaluronic acid derivatives; the process for their preparation and their use in the pharmaceutical, biomedical, surgical and healthcare fields.

INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C08B37/00 C08B11/12 A61K31/715 C08B37/06 C08B37/10

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Minimum documentation searched (classification system followed by classification symbols)
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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, CHEM ABS Data, WPI Data, PAJ, COMPENDEX

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Radke, M

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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